THE RELATIONSHIP OF SALINITY TO LARVAL SURVIVAL AND DEVELOPMENT IN NASSARIUS OBSOLETUS (GASTROPODA) [1,2]

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Not until recently has the relationship of salinity to the survival and development of marine larvae been seriously examined through laboratory observations. The earliest studies upon mollusks were confined almost exclusively to the embryonic and very early shelled pelagic stages of pelecypods. Thus, Seno, Hori and Kusakabe (1926), Amemiya (1926), and Rao (1951) determined the effect of reduced salinities upon the early development of, respectively: *Ostrea gigas* and *Crassostrea virginica; Ostrea angulata* and *Ostrea edulis;* and *Ostrea madrasensis.* Because the early larvae of *Crassostrea virginica* survived salinities far lower than those found in the habitat of the adult, Clark (1935) concluded that the effect of salinity was unimportant in determining the mortality of oyster spat in Malpeque Bay, P.E.I., Canada. Turner and George (1955) showed experimentally that the larvae of *Venus mercenaria* would not swim past a salinity discontinuity of 20 and 15 parts per thousand (‰). The very interesting results of Haskin (1964) reveal a direct relationship between salinity and swimming activity of oyster larvae (*Crassostrea virginica*). Haskin also demonstrated that light intensity and its spectral composition modify the response elicited from late "eyed" oyster larvae. Wells (1961) has compared the "salinity death points" of the adults and early larvae of two species of gastropods, *Thais floridana* and *Cerithium floridanum.* In the former, little difference between the "salinity death point" of the adult and larva was found, whereas in the latter species the "salinity death point" of the larva was at a higher salinity than of the adult. Not until the investigations of Davis (1958) upon *Crassostrea virginica* and *Venus mercenaria* and of Davis and Ansell (1962) on *Ostrea edulis* have observations on the effect of salinity over the entire pelagic period of larval molluscan development been made. Stickney (1964) in addition has recently cultured *Mya arenaria* larvae and noted their response to salinity. No laboratory experiments on the relationship between salinity and growth in estuarine prosobranch gastropod veliger larvae have been published. Field studies on the effect of salinity upon survival and development of larval mollusks deal largely with commercially important species (Nelson and Perkins, 1930; Carriker, 1951; Korringa, 1952; Kunkle, 1957; Haskin, 1964; etc.) and no attempt to summarize this work is made here.

A common species which inhabits the intertidal flats of estuaries along the east coast of North America from Chaleur Bay in the Gulf of St. Lawrence to northern Florida is the mud snail or basket shell, *Nassarius obsoletus* Say. The ecology, certain aspects of which have recently been reviewed (Scheltema, 1964), is rather well known and the pelagic larval development and early post-larval life history have been described (Scheltema, 1962a). The lower limits of salinity at which the adults of *N. obsoletus* are naturally found range between 15‰ and 20‰.

Though much is known about this ubiquitous species, nothing has yet been reported on the effect of salinity upon survival and growth of the veliger larvae. I report here upon the results of some experiments with the larvae of *N. obsoletus* which (1) demonstrate the lower limit at which salinity becomes lethal to both the larvae and adults, and (2) show the effect of reduced salinity on growth between the time of emergence from the egg capsule to the completion of larval development.

Experiments on the Lower Lethal Salinity for Nassarius obsoletus

The lethal salinity for a species may be determined either experimentally in the laboratory or from observations in the field. In the laboratory, organisms may be subjected to different salinities and their behavioral or physiological responses measured (*e.g.,* Blum, 1922), while in the field unusual natural conditons, such as sudden changes in runoff, may, by large mortalities, show when salinity limits tolerated by a particular species have been surpassed (*e.g.,* Beaven, 1946).

A criterion to be used in the laboratory by which the effect of salinity upon an organism may be quantitatively measured is difficult to find. The methods adopted here were unsophisticated, but the results were reasonably reproducible.

Salinity lethal to adult snails of *N. obsoletus* was determined by placing either 15 or 20 organisms from a collection made on the intertidal flats in one of a series of four-liter tanks. Salinities in the series systematically descended in value from full-strength sea water to about 5‰. Reduced salinities were obtained by diluting sea water with tap water. The resulting salinity in each tank was checked with a hydrometer. The interval between tanks in the initial experiments was 5‰. In subsequent experiments this was reduced to 2‰ as values approached the lethal limit of the organisms. All the experiments were performed at room temperature (*ca.* 20° C.). The animals were collected from a brackish-water estuary near the laboratory and held overnight at a salinity of 18‰ before use. At the beginning of the experiment the snails were directly transferred to the salinity being tested. The effect of salinity was appraised by watching the behavior of the snails. Snails completely withdrawn into the shell were considered "inactive," whereas those not completely withdrawn were regarded as "active." At each observation the percentage of active and inactive snails was recorded.

The results of these simple experiments are shown in Figure 1. The graph illustrates that the region of stress lay between 12.5‰ and 13.5‰ salinity. The results are after four hours, but when the experiments were extended over a period of three days the values do not differ significantly. A slight increase in the percentage activity was evident, but the lower lethal limit was not markedly shifted, nor was the value at which stress was first observed altered.

The lower lethal limit of salinity among veliger larvae of *N. obsoletus* was determined by methods similar to those used for the adult snails. The criterion used to determine the effect of salinity upon the larvae was their swimming activity. Fifty larvae shortly after their emergence from the egg capsule were placed in 400 ml. of sea water, the salinity of which was adjusted by the addition of tap water. The salinities tested ranged between 8.5‰ and 33.0‰ and were spaced at intervals of roughly 6‰ in the earlier and 3‰ in the later experiments. The temperature throughout was between 25° and 26° C., which is near the optimum for growth.

FIGURE 1. Inhibition of activity in adult *Nassarius obsoletus* resulting from reduced salinity at about 20° C. The graph illustrates the percentage snails active following a four-hour exposure period.
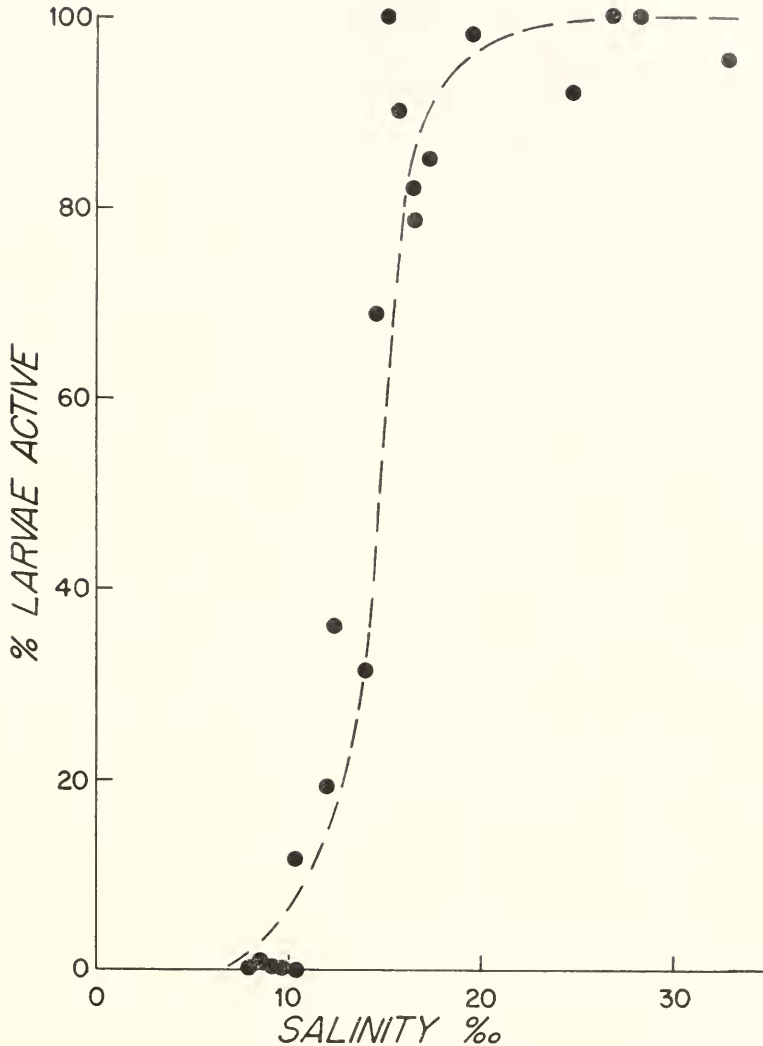
FIGURE 2. Inhibition of swimming in larvae of *Nassarius obsoletus* resulting from reduced salinity at 25–26° C. The larvae are those taken shortly after their emergence from the egg capsule. The results are following a 10-hour exposure period.

Larvae for all the experiments originated from water of 32‰ salinity. At the end of 10 hours the dish containing the larvae was placed under a bright light and those swimming were counted. The results of these experiments are shown in Figure 2. The greatest decrease in the percentage of swimming larvae falls between 14‰ and 15.5‰ salinity. Below 10‰ no larvae were ever seen swimming after the 10 hours of exposure.

The response of the late stage creeping-swimming larva of *N. obsoletus* was compared with that of the early veliger just after its emergence from the egg capsule.

These further experiments differed only in detail from those already described. Five-centimeter petri dishes were filled with dilutions of sea water ranging from 6.6‰ to 33.0‰ and in each, 10 veligers were placed. Into one sequence of dilutions were pipetted the early larvae; into the other, veligers which had completed their development to the creeping-swimming stage. The latter were reared in the laboratory for 19 days at 24° C. according to the method already described (Scheltema, 1962a).

TABLE I

Activity indexes of Nassarius obsoletus veliger larvae as a function of salinity*

	Early pelagic larvae (1–3 days after emergence from egg capsule)				
Salinity	1 min.	20 min.	1 hour	3 hours	Mean for 3 hours
6.6	0	1	0	0	0.3
9.0	0	0	1	1	0.5
11.0	0	1.5	1	2	1.1
13.2	0	2	2	3	1.8
16.5	1	3	3	3	2.5
19.8	3	4	4	4	3.8
26.4	4	4	4	4	4.0
33.0	4	4	4	4	4.0
	Creeping-swimming stage**				
6.6	0.0	0.0	0.0	0.0	0.0
9.0	0.4	0.0	0.0	0.0	0.1
11.0	0.4	0.0	0.0	1.0	0.4
13.2	0.0	0.4	0.2	2.6	0.8
16.5	3.0	2.8	3.4	3.9	3.3
19.8	3.0	2.0	3.8	4.0	3.2
26.4	4.0	4.0	3.9	3.5	3.9
32.0	4.0	4.0	3.9	3.5	3.9

* The definition of this term is given in the text.
** The values in these experiments were based on evaluation of each individual larva's performance, while those of the early larvae were simultaneously estimated by assigning a value to all the larvae in the dish.

The results of these experiments were expressed in terms of "activity indexes" which were recorded at the beginning of the experiment and after 20 minutes, one hour and three hours. The following numerical values were used to describe the responses of the larvae and to compute the "activity indexes": 0, no movement of velar cilia; 1, cilia of velum moving but not vigorously enough to allow the larva to swim; 2, larva moving sluggishly along the bottom and sides of dish or, if creeping-swimming stage, then responding immediately to the touch of a pin; 3, actively swimming or creeping; and 4, very actively swimming or creeping. All examinations were made under bright light.

Results of one such experiment are summarized in Table I. A mean activity index of between 2.5 and 4.0 indicates the "normal" range of behavior. The

activity indexes of the new and old larvae as a function of salinity have been plotted together in Figure 3. This graph shows that there is no significant difference in the activity of the two ages of larvae relative to the salinity. A sharp decrease in activity occurred between 13.5‰ and 16.5‰ salinity. Similar experiments of longer duration (38 hours) fully confirmed the results shown here.
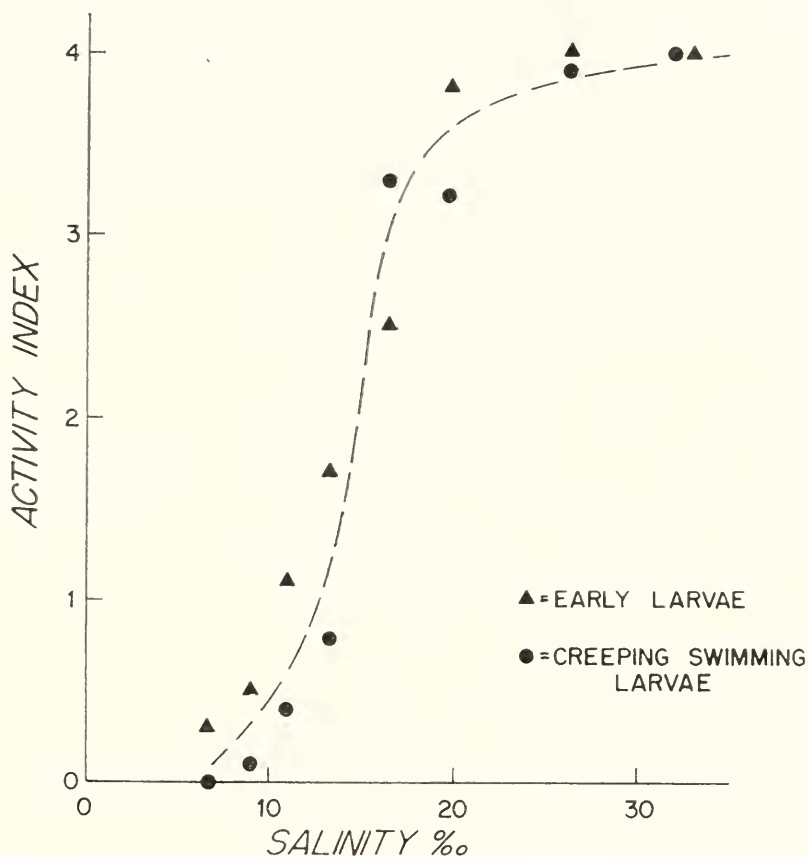

FIGURE 3. Relationship of the "activity index" of early and creeping-swimming larvae of *Nassarius obsoletus* to salinity. The graph illustrates the average "activity index" resulting from a series of observations over a period of three hours.

EXPERIMENTS ON THE EFFECT OF SALINITY UPON LARVAL GROWTH OF NASSARIUS OBSOLETUS

The larval life of *Nassarius obsoletus* can be divided into two periods. The first of these is a phase of rapid growth and external development leading to the creeping-swimming or veliconcha stage (Scheltema, 1962a). During this period the growth rate is essentially constant (Fig. 4). This is followed by a second period of very slow growth and no apparent further external morphological change. The beginning of the second period is evident from the completion of the develop-

ment of the foot and from the behavior of the larvae, namely, frequent creeping on, and inspection of, the bottom .

The length of the first period is determined by those conditions which control larval growth. The length of the second period varies greatly, at least two-fold in *N. obsoletus,* and depends upon the encounter by the larva of a sediment suitable for post-larval life (Scheltema, 1961).   Under favorable conditions metamorphosis may occur very near the beginning of the second period.   The total length of
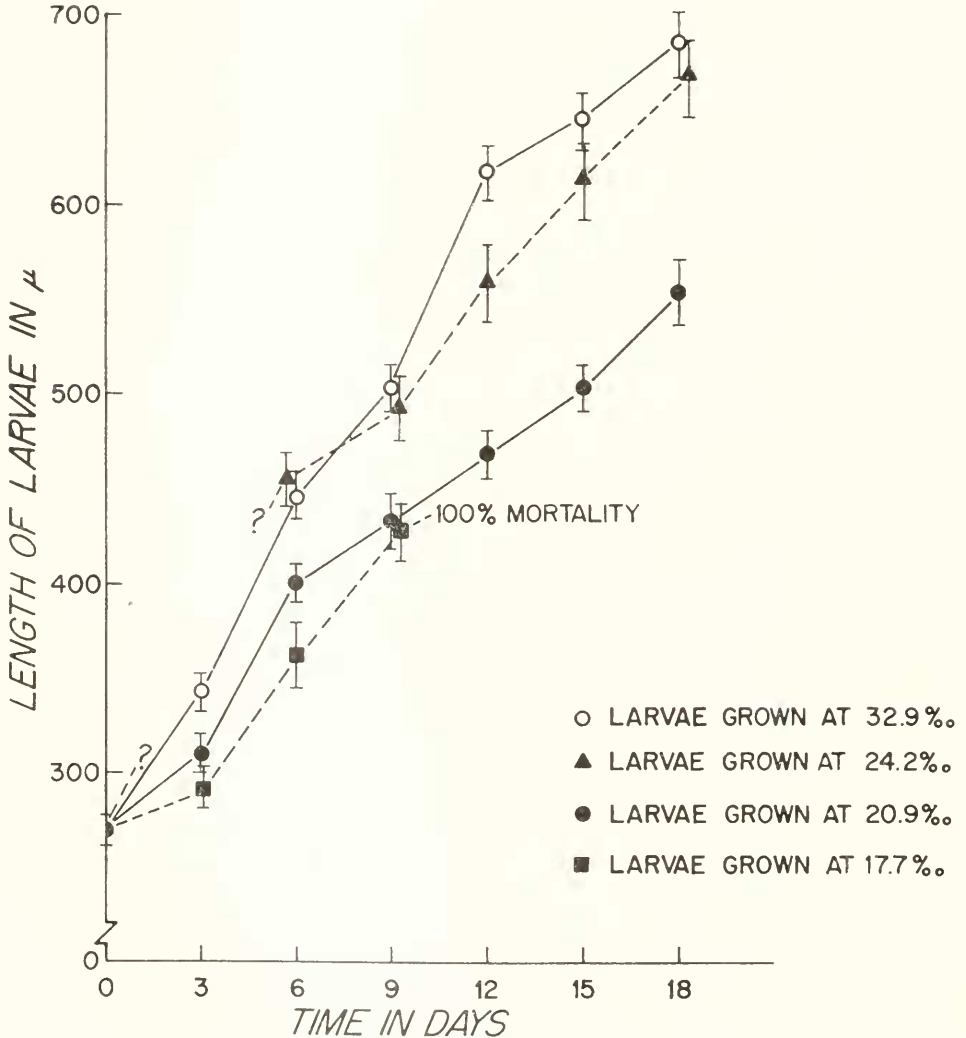

FIGURE 4.   Increase in length of *Nassarius obsoletus* veliger larvae from the time of emergence from egg capsules to the completion of their development to the creeping-swimming stage.   This is the first pelagic phase, during which growth occurs at a rapid and nearly constant rate.   Each curve shows the result at a different salinity as indicated by the conventions.

larval life consequently is not determined solely by the growth rate, but also by the opportunity for metamorphosis upon a favorable substratum. In investigating the effect of salinity on the larvae, I have confined my attention only to the period of rapid and constant growth rate.

Laboratory experiments on the growth rate of veliger larvae are possible only after techniques for their mass culture are worked out (Scheltema, 1962a). The veliger larvae of *N. obsoletus* were grown in a series of 10-liter vessels with salinities ranging from that minimal for survival to that of full-strength sea water. It was soon found that the larvae held at a salinity of less than 16‰ did not survive more than a few days. Consequently the minimum average salinity in the experiment reported here was 17.7‰ and the remainder of the series had salinities of 20.9‰, 24.2‰, 26.2‰ and 32.8‰. All larval cultures were fed the euryhaline diatom, *Phaeodactylum tricornutum,* from the same algal culture.

TABLE II

Length in microns of Nassarius obsoletus throughout larval development as a function of the salinity

Mean salinity ‰	Age in days						
	0	3	6	9	12	15	18
17.7	278 ± 4*	291 ± 4	363 ± 8	429 ± 7	—	—	—
20.9	278 ± 4	312 ± 6	401 ± 6	433 ± 7	468 ± 6	501 ± 6	552 ± 8
24.2	278 ± 4	—	458 ± 7	492 ± 8	556 ± 9	613 ± 10	665 ± 10
26.2	278 ± 4	—	447 ± 6	487 ± 9	543 ± 8	586 ± 6	—
32.9	278 ± 4	343 ± 4	446 ± 5	502 ± 8	615 ± 8	643 ± 8	683 ± 9

* One standard error is indicated on all values of this table.

The mean temperature of the veliger cultures during this experiment, which extended over a period of 18 days, was 23.1° C. The maximum difference in water temperature measured during the course of the experiment was 1.1° C. However, since all cultures were kept together in the same temperature bath, the same fluctuations were experienced by all. Maximum temperature differences between cultures were 0.5° C., but the mean difference was only 0.2° C.

At the lower values, the salinity in the cultures never varied more than ±0.5‰ from the mean; in cultures at salinities higher than 24‰, the maximum deviation from the mean value never exceeded ±0.7‰. Growth of the larvae was determined by measuring the maximum shell dimensions at three-day intervals with an ocular micrometer at a magnification of 100×. This measurement, hereafter termed *length,* was made on an aliquot of 30 larvae from each culture. Previous measurements have shown this to be an adequate sample size.

The results of the experiment (Table II) show that among salinities above 24‰ there is no statistically significant difference in length. As the salinity reached 21.0‰, the difference becomes significant. Below 21.0‰, the data indicate substantial decrease in shell growth rate. Under the conditions of the experiment, completion of larval development to metamorphosis did not occur at a salinity of 17.7‰.

The pertinent data are summarized by means of growth curves in the graph of Figure 4. Here the vertical lines indicate two standard errors. The difference between the two curves above 21‰ with those of 21‰ and less is quite evident, and the statistical significance is conspicuous.

The results of two additional experiments, similar to the one just described above, together with the data from the first experiment, are summarized in Table III. In each of the additional experiments, two cultures of veligers were started simultaneously with larvae obtained randomly from the same collection of egg capsules. The initial size of the larvae in the two cultures of an experiment were consequently the same. Development previous to emergence of larvae from the

TABLE III

Percentage inhibition of growth in Nassarius obsoletus larvae resulting from the lowest salinity at which development is completed to metamorphosis

Expt. no.	Temp. range ° C.	Age of larvae at end of expt.	Length μ at begin expt.	A			B			Percent-age inhi-bition (1)
				Mean salinity ‰	Length μ at end expt. (A)	Total growth* (ΔA)	Mean salinity ‰	Length μ at end expt. (B)	Total* growth (ΔB)	
I	23.0–24.0	18	278	32.9	683±9***	405	20.9	552±8	274	32.1
II	19.5–21.3	19	270**	33.1	701±4	431	21.5	634±6	364	15.5
III	26.1–28.0	12	249	33.2	545±5	296	21.3	504±8	255	13.8
									Sum	61.4
									Mean	20.5

* This denotes the difference between the initial length at the time of emergence from the egg capsule and the length at the end of the experiment.

** In this experiment only the length of the larvae at the termination of the experiment is known; the assumed length of 270 μ is the usual length of larvae at the time of emergence from the egg capsule. However, even if extreme values are assumed, the percentage difference in the final column is altered by no more than ±1%.

*** One standard error is indicated.

egg capsules was at room temperature. The unusually small initial size of the larvae in experiment III is a peculiarity of that particular collection of egg capsules and is probably related to the time at which they were deposited within the breeding cycle of the female snails.

In one of the two cultures in each experiment, larvae were grown at the salinity of normal sea water. This culture is designated as "A" in each of the experiments of Table III. In the other culture, designated "B" in Table III, the larvae were grown at a reduced salinity near 21‰. Both cultures in each experiment were terminated at the same time. This was done near the end of the period of constant growth rate in the high salinity culture "A" of each experiment.

The percentage inhibition, I, due to the reduction of salinity is shown for each experiment in the right-hand column of Table III and was computed by the relationship

$$I = \frac{\Delta A - \Delta B}{\Delta A} \times 100,$$

where ΔA is the change in length of shell between the beginning and end of the experiment among larvae maintained at sea-water salinities, and ΔB is the change in shell length of larvae held at a minimum salinity required for completion of development. Table III shows that maximum inhibition was obtained at a temperature range of between 23° and 24° C., which is near the optimum for growth of the larvae of *N. obsoletus* (Scheltema, 1963). At both higher and lower temperature ranges there was substantially less inhibition in growth. However, owing to the large differences in larval growth rate frequently obtained between experiments, a direct effect or interaction of temperature on growth inhibition by reduced salinity cannot be assumed without further experiments. The results show that at the lowest salinity at which development to metamorphosis was completed, an average of 20.5% inhibition of growth occurred.

Discussion

Salinity as a limiting factor to distribution

The upstream distribution of most organisms that live within estuaries is seemingly related to salinity. However, mere correspondence between salinity values and the distribution of a particular species cannot in itself be taken as sufficient evidence that salinity is limiting. To show this, it is necessary to discover the extremes tolerated by an organism *throughout* its life history.

Thorson (1946, p. 472) has suggested that the larval stage may limit the distribution of bottom species, as this stage is "the weakest link of the chain." Experiments upon the salinity lethal to larval and adult *N. obsoletus,* however, showed no large difference between their lower tolerances. Such differences which did appear might be accounted for by the previous acclimation of the adult snails to a lower salinity or by the inadequacy of the techniques in making such small distinctions. The lower lethal salinity did not change significantly as larval development progressed (Figs. 2 and 3).

The known upstream distribution of *N. obsoletus* into estuaries in many instances seems to correlate well with the lower lethal salinity determined in the laboratory. Hence, Pfitzenmeyer (1961) found the species at locations in Chesapeake Bay where the summer salinity was 14.6‰. On the other hand, in certain estuaries on Cape Cod snails do not ascend farther up than a summer bottom salinity of 17‰, and in such instances salinity is probably not the factor limiting distribution. In order to make valid comparisons, laboratory results must be related to *bottom* salinities in areas where the seasonal extremes are known.

Salinity and larval growth rate

Growth of *N. obsoletus* is inhibited only as the lower limit of salinity tolerance is approached (Scheltema, 1962b). Thus, it was not until the salinity was near 20‰ that any significant effect on growth rate was noticed. Although not directly investigated, the decreased rate of growth in *N. obsoletus* is not likely to be related in any simple way to osmotic activity because marine mollusks, insofar as known, have no active osmotic control involving the expenditure of energy (Prosser *et al.,* 1961). The mortality of larvae at salinities below 20‰ was high.

No larvae survived beyond the intermediate stage of development in laboratory culture, although growth proceeded up until the ninth day at 17.7‰ salinity. The inhibition of growth attributable to the reduction of salinity amounted on the average to about 20%.
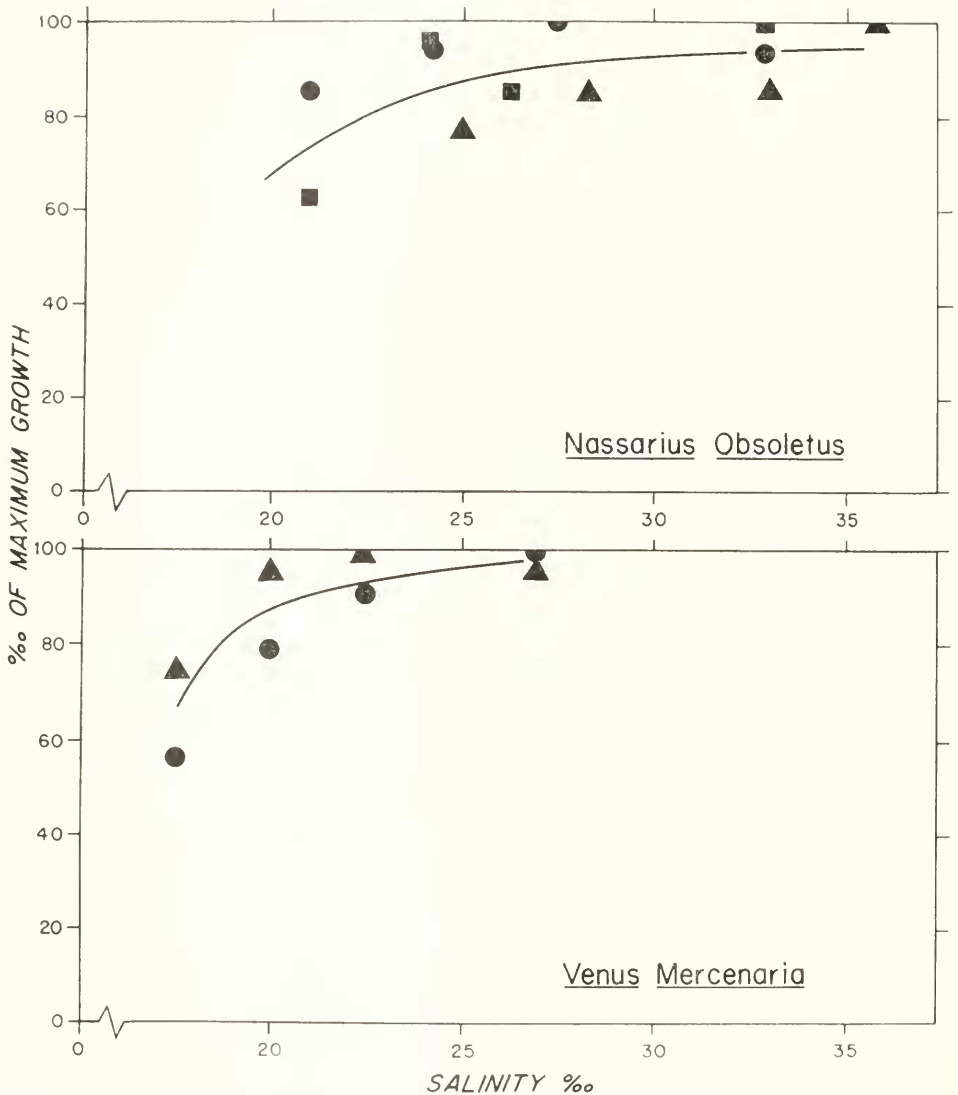

FIGURE 5. Percentage growth obtained at various reduced salinities relative to the maximum growth obtained at optimal salinity conditions in the gastropod, *Nassarius obsoletus* Say and pelecypod, *Venus mercenaria* L. Data for *V. mercenaria* are from Davis (1958), p. 301, Fig. 1. Growth data for each species were used only if more than 5% of the larvae in the culture completed development to metamorphosis. The data refer to 15 days after the beginning of planktotrophic life in *N. obsoletus* (*i.e.*, creeping-swimming stage) and 12 days after fertilization in *V. mercenaria*. The different conventions indicate values for individual series of experiments. The curves are only intended to be suggestive.

TABLE IV

Maximum differences of larval growth rate attributable to various ecological factors
(Average values of I from experimental data)

	N. obsoletus	V. mercenaria
Physical characteristics		
Temperature		
Range within which development is completed	17.5° to 30° C.*	18° to 30° C.**
Average difference in growth rate between optimum and minimum required for complete development	50%*	60%**
Salinity		
Range within which development is completed	>17.7‰	>15.0‰***
Average difference in growth rate between optimum and minimum required for complete development	20%	34%***
Extreme difference in growth rate observed between optimum and minimum required for complete development	13 to 40%	22 to 45%***
Biological characteristics		
Concentration of algal food		
(Differences between optimum and minimal growth obtained at concentrations between 2.5×10^{-3} and 40×10^{-3} mm.³ packed cells per 3-l. culture)		
Isochrysis galbana	—	17.7%****
Monochrysis lutheri	—	51.2%****
Chlorella sp. (causes inhibition of growth and death of larvae at highest concentrations)	—	29.7%****†
Phaeodactylum tricornutum (data from relative concentrations only)	ca. 50% (preliminary estimation)	—
Species of algal food		
(based on 10 species of or combination of species when equal packed cell volumes were used)	—	Up to 75%††

* Scheltema (1963), p. 17, Fig. 2.
** Loosanoff *et al.* (1951), p. 71, Table III; Loosanoff (1959), p. 315, Fig. 6.
*** Davis (1958), p. 301, Fig. 1.
**** Davis and Guillard (1958), p. 302, Fig. 6.
† This figure represents difference in growth at concentrations between 2.5×10^{-3} and 20×10^{-3} mm.³ per 3-liter culture. At higher concentrations larvae did not survive.
†† Davis and Guillard (1958), p. 298, Fig. 3; p. 299, Fig. 4.

Because differences in the nutritional value of the algal food cells used in growth experiments are not readily controlled (Walne, 1963), it is not possible to compare the results from one series of experiments directly with the next without elaborate experimental procedures. A direct comparison between most series of growth experiments usually shows large discrepancies. Only cultures of larvae simultaneously grown using the same source of algal food can be directly compared with one another. It is possible, however, to compare differences in computed growth rates. Likewise the per cent inhibition, *I,* computed from the equation given above, can be directly compared if the differences in length are derived

from samples of similar size. Using this kind of information it is also possible to compare the relative importance of reduced salinity to growth between species of mollusks. In Figure 5 this has been done for two species which show similar distributions within estuaries along the Atlantic coast of the United States: the data of Davis (1958) on the growth of larvae of the pelecypod, *Venus mercenaria,* are compared with growth data from the larvae of *N. obsoletus.* The conclusion may be made that salinity little affects larval growth in either species until the lower limit of salinity tolerance is approached. Only the lower third of the salinity range has any marked inhibitory effect on growth and there is no simple linear relationship between growth and the salinity level. The similarity in response of the two species, *V. mercenaria* and *N. obsoletus,* is striking.

The importance of salinity relative to other factors affecting larval growth

An indication of the relative importance of salinity to larval growth can be had by comparing its maximum effect relative to that of some other factors known to control growth rate of *N. obsoletus* and *V. mercenaria.* By tabulating the values for maximum percentage difference obtained from that of optimum growth, the importance of various ecological factors in limiting growth rate becomes apparent. This is shown in Table IV. Here the range within which completion of development occurs is given for physical characteristics of the environment, and the percentage values are average maximum differences in growth attributable to these physical factors. Differences in growth rate under different biological conditions for which data are available, *viz.* concentration and species of algal food, are given within the limits of concentrations indicated. The maximum inhibition of growth varies with the algal species. On the basis of the figures given, the concentration and the species of algal food usually affect growth rate of *V. mercenaria* much more than any of the physical factors of the environment. There is preliminary evidence that this is also true for *N. obsoletus* larvae. The food value of algal cells to mollusk larvae in relation to the conditions under which the algal cells were grown is not yet known (see Walne, 1963). The table shows that the importance of low salinity in inhibiting growth rate, within the limits in which development of the larvae is completed, is certainly minimal, less than any other factor in the environment known to retard growth of the larvae.

This work is dedicated to the memory of G. Francis Beaven, who in his quiet way first interested me in the relationship between salinity and the distribution and survival of estuarine organisms.

SUMMARY

1. There is no large difference between the lower lethal salinity for the veliger larva and the adult of *Nassarius obsoletus.* The region of stress in snails is between 12.5‰ and 13.5‰; that of the early larva is between 14.0‰ and 15.5‰. Throughout larval development no change occurred in the value of the lower lethal salinity.

2. Difference in growth rate of *N. obsoletus* larvae observed at salinities above 24‰ are usually slight. However, at 21‰ and less there is a statistically sig-

nificant drop in growth rate, while at a mean salinity of 17.7‰, it was not possible to rear the larvae to the completion of development and metamorphosis. The maximum inhibition of growth attributable to the affects of salinity, within the range at which development of the larvae is completed, is between approximately 13% and 40% and averages about 20%. This is less than that of other ecological factors known to retard growth.

3. The net result of reduced salinity, within the lower third of the range at which the larval development of *N. obsoletus* is completed, is an increase in the length of time to reach the creeping-swimming stage which precedes metamorphosis, and an increased mortality of larvae as the limit of salinity tolerance is reached.

4. A comparison of data from *N. obsoletus* with that of another molluscan species, the pelecypod *Venus mercenaria*, which is found at approximately the same salinities within Atlantic coast estuaries along the United States, shows striking similarities.

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